CLAIMS:

1. A process for the preparation and identification of hydrolase mutants having improved properties with respect to stereo- or regioselectivity, catalytic activity or stability, characterized in that

a) a starting hydrolase gene is mutagenized by a modified polymerase chain reaction (PCR), wherein the mutation rate and total number of mutations in the amplified DNA is adjusted by adjusting the concentrations of Mg^{2+}, Mn^{2+} and of the deoxynucleotides and by adjusting the number of cycles; and/or

b) one or more starting hydrolase genes, one or more hydrolase genes mutated according to step a), or mixtures of one or more starting hydrolase genes and one or more hydrolase genes mutated according to step a) are mutagenized by enzymatically fragmenting said genes, followed by enzymatic reassembly of the fragments produced to give complete recombinant hydrolase genes;

c) the mutated hydrolase genes obtained according to step a) or b) are transformed into a host organism; and

d) hydrolase mutants having improved properties, expressed by transformants obtained in step c), are identified by a test method.

2. The process according to claim 1, wherein an average mutation rate of 1-2 base substitutions, per one hydrolase gene to be mutagenized, is adjusted in the PCR in step a) by adjusting the concentrations of Mg^{2+}, Mn^{2+} and of the deoxynucleotides.

3. The process according to claim 1, wherein a hydrolase gene mutagenized in a PCR previously performed according to claim 1 is used as the starting hydrolase gene in step a).

4. The process according to claim 1, wherein the enzymatic fragmentation of the hydrolase genes in step b) is performed using a deoxyribonuclease.

5. The process according to claim 1, wherein the reassembly of the fragments in step b) is effected enzymatically by means of a thermostable DNA polymerase using temperature cycles in which the parameters of temperature and duration of cycles are adjusted.

6. The process according to claim 1, wherein the mutation rate is adjusted during the enzymatic reassembly in step b) by adjusting the concentrations of Mg^{2+}, Mn^{2+} and of the deoxynucleotides.

7. The process according to claim 1, wherein the completely recombined hydrolase genes are amplified by a polymerase chain reaction in step b) after completion of the reassembly reaction.

8. The process according to claim 1, wherein either modified hydrolase genes obtained from step a) according to claim 1 or 2 or several hydrolase genes mutagenized according to claim 3 are subjected to fragmentation and reassembly in step b).

9. The process according to claim 1, wherein synthetically prepared gene fragments are additionally used for the reassembly in step b).

10. The process according to claim 1, wherein hydrolase gene fragments from different organisms sharing a sequence homology of at least 60% can be used for the reassembly in step b).

11. The process according to claim 2 or 6, wherein the hydrolase mutants are lipase or esterase mutants, and the concentration of the magnesium ions is from 1.5 to 8.0 mM, preferably from 5.8 to 6.4 mM, and the concentration of the manganese ions is from 0.0 to 3.0 mM, preferably < 0.3 mM.

12. The process according to claim 2 or 6, wherein the hydrolase mutants are lipase or esterase mutants, and the concentration of the deoxynucleotide triphosphates is from 0.05 to 1.0 mM, preferably 0.2 mM.

13. The process according to claim 1, wherein for the test for stereo- or regioselectivity of the hydrolase mutants in step d), a test substrate is provided with a chromophorous group which causes a spectrometrically determined change of absorption or emission upon cleavage by the catalyst, and equal amounts of the hydrolase mutants are added to the pure stereo- or regioisomers of the test substrate in separate test vessels, and the stereo- or regioselectivity can be determined from the ratio of the linear initial reaction rates obtained.

14. The process according to claim 13, wherein the stereo- or regioisomers of a compound with a UV/VIS-active or fluorescence-active molecular group bound through a carboxylic acid ester or carboxylic acid amide linkage are used as the test substrate.

15. The process according to claim 14, wherein said UV/VIS-active molecular group is a p-nitrophenyl residue.

16. The process according to claim 1, wherein the test for stereo- or regioselectivity in step d) is effected through determination of the change of concentration with time of free fatty acids or succinic acid, wherein the corresponding stereo- or regioisomeric carboxylic acid

esters or amides are hydrolyzed in separate vessels by means of the hydrolase mutants to give free fatty acids or succinic acid.

17. The process according to claim 1, wherein the test for stereo- or regioselectivity in step d) is effected through measuring the radioactivity, wherein the hydrolase mutants are reacted with stereo- or regioisomers having different radioactive labels in one functional group, and wherein the mixture of the stereo- or regioisomers is fixed on a support.

18. The process according to claim 17, wherein one of the stereo- or regioisomers of the support-bound mixture of isomeric compounds is labeled with the radioisotope ^{3}H, and the other stereo- or regioisomer is labeled with the radioisotope ^{14}C.

19. The process according to claim 1, wherein the test for stereoselectivity in step d) is effected through the capillary-electrophoretic determination of the reaction products and educts of a test reaction, the separation of the stereoisomeric reaction products and educts being performed in chirally modified capillaries.

20. The process according to claims 13 to 19, wherein several reactions are performed in parallel in microtitration plates.

21. The process according to claim 1, wherein the position of the codon coding for the changed amino acid is localized by sequencing in the mutants having improved properties identified in step d), followed by generating a set of hydrolase genes with all possible codons for this position by means of site-directed saturation mutagenesis, and the mutated hydrolase genes thus obtained are further treated in analogy with steps c) and d) of claim 1.

22. The process according to claim 21, wherein the localization of the position of the codon coding for the changed amino acid is effected through DNA sequencing.

23. A hydrolase mutant obtainable by a process according to one or more of claims 1 to 22.

24. The hydrolase mutant according to claim 23 which is a lipase mutant.

25. The hydrolase mutant according to claim 23 which is an esterase mutant.

26. The hydrolase mutant according to claim 24 which is a lipase mutant of the starting lipase from the strain *P. aeruginosa*.

27. The hydrolase mutant according to claim 26 which is obtainable by expression from the transformants P1B 01-E4 (DSM 11 658), P2B 08-H3 (DSM 11 659), P3B 13-D10 (DSM 11 660), P4B 04-H3 (DSM 12 322), P5B 14-C11 (DSM 12 320) or P4BSF 03-G10 (DSM 12 321).

28. The hydrolase mutant according to claim 24 which has the amino acid sequence of the mature proteins shown in SEQ ID NOS. 4, 6, 8, 12, 14, 16 or 18.

29. A DNA sequence coding for a hydrolase mutant according to one or more of claims 23 to 28.

30. The DNA sequence according to claim 29 which comprises a DNA sequence shown in SEQ ID NOS. 3, 5, 7, 11, 13, 15 or 17.

31. A vector comprising a DNA sequence according to claim 29 or 30.

32. A transformant comprising a DNA sequence according to claim 29 or 30 and/or a vector according to claim 31.

33. The transformant according to claim 32 which is transformant P1B 01-E4 (DSM 11 658), P2B 08-H3 (DSM 11 659), P3B 13-D10 (DSM 11 660), P4B 04-H3 (DSM 12 322), P5B 14-C11 (DSM 12 320) or P4BSF 03-G10 (DSM 12 321).

34. A process for the preparation of hydrolase mutants having improved properties, comprising culturing a transformant according to claim 32 or 33.

35. A method for testing catalysts for stereo- or regioselectivity, wherein equal amounts of the catalyst are added to a test substrate and to the pure stereo- or regioisomers of the test substrate, provided with a chromophorous group which causes a spectrometrically determinable change of absorption or emission upon cleavage by the catalyst, in separate test vessels, and the stereo- or regioselectivity is determined from the ratio of the linear initial reaction rates obtained.